NESTED OLIGONUCLEOTIDES CONTAINING A HAIRPIN FOR NUCLEIC ACID AMPLIFICATION

Related Applications

This application claims priority to U.S. Provisional Application No. 60/254,669 filed December 11, 2000 and to U.S. Provisional Application No. 60/323,400 filed September 19, 2001. The disclosures of both these Provisional Applications are incorporated herein in their entirety by this reference.

Technical Field

This disclosure relates to engineered templates useful for amplification of a target nucleic acid sequence. More specifically, templates which are engineered to contain a predetermined sequence and a hairpin structure are provided by a nested oligonucleotide extension reaction. The engineered templates allow Single Primer Amplification (SPA) to amplify a target sequence within the engineered template. In particularly useful embodiments, the target sequences from the engineered templates are cloned into expression vehicles to provide a library of polypeptides or proteins, such as, for example, an antibody library.

Background of Related Art

Methods for nucleic acid amplification and detection of amplification products assist in the detection, identification, quantification, isolation and sequence analysis of nucleic acid sequences. Nucleic acid amplification is an important step in the construction of libraries from related genes such as, for example, antibodies. These libraries can be screened for antibodies having specific, desirable activities. Nucleic acid analysis is important for detection and identification of pathogens, detection of gene alteration leading to defined phenotypes, diagnosis of genetic diseases or the susceptibility to a disease, assessment of gene expression in development, disease and in response to defined stimuli, as well as the various genome projects. Other applications of nucleic acid amplification method include the detection of rare cells, detection of pathogens, and the detection of altered gene expression in malignancy, and the like. Nucleic acid amplification is also useful for qualitative analysis (such as, for example, the detection of the presence of defined nucleic acid sequences) and quantification of defined

gene sequences (useful, for example, in assessment of the amount of pathogenic sequences as well as the determination of gene multiplication or deletion, and cell transformation from normal to malignant cell type, etc.). The detection of sequence alterations in a nucleic acid sequence is important for the detection of mutant genotypes, as relevant for genetic analysis, the detection of mutations leading to drug resistance, pharmacogenomics, etc.

There are many variations of nucleic acid amplification, for example, exponential amplification, linked linear amplification, ligation-based amplification, and transcription-based amplification. One example of exponential nucleic acid amplification method is polymerase chain reaction (PCR) which has been disclosed in numerous publications. See, for example, Mullis et al. Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); Mullis K. EP 201,184; Mullis et al. U.S. Pat. No. 4,582,788; Erlich et al. EP 50,424, EP 84,796, EP 258,017, EP 237,362; and Saiki R. et al. U.S. Pat. No. 4,683,194. In fact, the polymerase chain reaction (PCR) is the most commonly used target amplification method. PCR is based on multiple cycles of denaturation, hybridization of two different oligonucleotide primers, each to opposite strand of the target strands, and primer extension by a nucleotide polymerase to produce multiple double stranded copies of the target sequence.

Amplification methods that employ a single primer, have also been disclosed. See, for example, U.S. Pat. Nos. 5,508,178; 5,595,891; 5,683,879; 5,130,238; and 5,679,512. The primer can be a DNA/RNA chimeric primer, as disclosed in U.S. Pat. No. 5,744,308.

Some amplification methods use template switching oligonucleotides (TSOs) and blocking oligonucleotides. For example, a template switch amplification in which chimeric DNA primer are utilized is disclosed in U.S. Pat. Nos. 5,679,512; 5,962,272; 6,251,639; and by Patel et al. Proc. Natl. Acad. Sci. U.S.A. 93:2969-2974 (1996).

However the previously described target amplification methods have several drawbacks. For example, the transcription base amplification methods, such as Nucleic Acid Sequence Based Amplification (NASBA) and transcription mediated amplification (TMA), are limited by the need for incorporation of the polymerase promoter sequence into the amplification product by a primer, a process prone to result in non-specific amplification. Another example of a drawback of the current amplification methods is the requirement of two binding events which may have optimal binding at different temperatures. This combination of factors results in increased likelihood of mis-priming and resultant amplification of sequences

other than the target sequence. Therefore, there is a need for improved nucleic acid amplification methods that overcome these drawbacks. The invention provided herein fulfills this need and provides additional benefits.

Summary

A method of amplifying nucleic acid has been discovered which includes the steps of a) annealing a primer to a template nucleic acid sequence, the primer having a first portion which anneals to the template and a second portion of predetermined sequence; b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the end of the template, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer; c) separating the polynucleotide synthesized in step (b) from the template; d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide, and a second portion having a hairpin structure; e) extending the polynucleotide synthesized in step (b) to provide a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence; and f) amplifying the extended polynucleotide using a single primer having the predetermined sequence.

In an alternative embodiment, the method of amplifying nucleic acid includes the steps of a) annealing a primer and a boundary oligonucleotide to a template nucleic acid sequence, the primer having a first portion which anneals to the template and a second portion of predetermined sequence; b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the portion of the template to which the boundary oligonucleotide anneals, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer; c) separating the polynucleotide synthesized in step (b) from the template; d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide and a second portion having a hairpin structure; e) extending the polynucleotide synthesized in step (b) to provide a portion that is complementary to the hairpin structure and a terminal portion that is complementary to the predetermined sequence;

and f) amplifying the extended polynucleotide using a single primer having the predetermined sequence.

In yet another embodiment, the method of amplifying nucleic acid includes the steps of a) annealing an oligo dT primer and a boundary oligonucleotide to an mRNA template; b) synthesizing a polynucleotide that anneals to and is complementary to the portion of the template between the location at which the first portion of the primer anneals to the template and the portion of the template to which the boundary oligonucleotide anneals, the polynucleotide having a first end and a second end, wherein the first end incorporates the primer; c) separating the polynucleotide synthesized in step (b) from the template; d) annealing a nested oligonucleotide to the second end of the polynucleotide synthesized in step (b), the nested oligonucleotide having a first portion that anneals to the second end of the polynucleotide, and a second portion having a hairpin structure; e) extending the polynucleotide synthesized in step (b) to provide an extended polynucleotide that includes a portion that is complementary to the hairpin structure and a poly A terminal portion; and f) amplifying the extended polynucleotide using a single primer.

In another aspect an engineered nucleic acid strand is disclosed which has a predetermined sequence at a first end thereof, a sequence complementary to the predetermined sequence at the other end thereof, and a hairpin structure therebetween.

In yet another aspect, a method of amplifying a nucleic acid strand has been discovered which includes the steps of providing an engineered nucleic acid strand having a predetermined sequence at a first end thereof, a sequence complementary to the predetermined sequence at the other end thereof and a hairpin structure therebetween, and contacting the engineered nucleic acid strand with a primer containing at least a portion of the predetermined sequence in the presence of a polymerase and nucleotides under conditions suitable for polymerization of the nucleotides.

Once the engineered nucleic acid is amplified a desired number of times, restriction sites can be used to digest the strand so that the target nucleic acid sequence can be ligated into a suitable expression vector. The vector may then be used to transform an appropriate host organism using standard methods to produce the polypeptide or protein encoded by the target sequence. In particularly useful embodiments, the techniques described herein are used to

amplify a family of related sequences to build a complex library, such as, for example, an antibody library.

Brief Description of Drawings

Fig. 1 is a schematic illustration of a primer and boundary oligo annealed to a template;

Fig. 2A is a schematic illustration of a restriction oligo annealed to a nucleic acid strand;

Fig. 2B is a schematic illustration of a primer annealed to a template that has a shortened 5' end;

Fig. 3 is a schematic illustration of a nested oligo having a hairpin structure annealed to a newly synthesized nucleic acid strand;

Fig. 4A is a schematic illustration of an engineered template in accordance with this disclosure; and

Fig. 4B is a schematic illustration of an engineered template in accordance with an alternative embodiment.

Figs. 5A-5C is a chart showing the sequences of clones produced in Example 4.

Figs. 6A-6C is a chart showing the sequences of clones produced in Example 7.

Detailed Description of Preferred Embodiments

The present disclosure provides a method of amplifying a target nucleic acid sequence. In particularly useful embodiments, the target nucleic acid sequence is a gene encoding a polypeptide or protein. The disclosure also describes how the products of the amplification may be cloned and expressed in suitable expression systems. In particularly useful embodiments, the techniques described herein are used to amplify a family of related sequences to build a complex library, such as, for example, an antibody library.

The target nucleic acid sequence is exponentially amplified through a process that involves only a single primer. The ability to employ a single primer (i.e., without the need for both forward and reverse primers each having different sequences) is achieved by engineering a strand of nucleic acid that contains the target sequence to be amplified. The engineered strand of nucleic acid (sometimes referred to herein as the "engineered template") is prepared from two templates; namely, 1) a starting material that is a natural or synthetic nucleic acid (e.g., RNA, DNA or cDNA) containing the sequence to be amplified and 2) a nested oligonucleotide that provides a hairpin structure. The starting material can be used directly as the original template, or, alternatively, a strand complementary to the starting material can be prepared and

used as the original template. The nested oligonucleotide is used as a template to extend the nucleotide sequence of the original template during creation of the engineered strand of nucleic acid. The engineered strand of nucleic acid is created from the original template by a series of manipulations that result in the presence of a predetermined sequence at one end thereof and a hairpin structure. It is these two features that allow amplification using only a single primer.

Any nucleic acid, in purified or nonpurified form, can be utilized as the starting material for the processes described herein provided it contains or is suspected of containing the target nucleic acid sequence to be amplified. Thus, the starting material employed in the process may be, for example, DNA or RNA, including messenger RNA, which DNA or RNA may be single stranded or double stranded. In addition, a DNA-RNA hybrid which contains one strand of each may be utilized. A mixture of any of these nucleic acids may also be employed, or the nucleic acids produced from a previous amplification reaction herein using the same or different primers may be utilized. The target nucleic acid sequence to be amplified may be a fraction of a larger molecule or can be present initially as a discrete molecule. The starting nucleic acid may contain more than one desired target nucleic acid sequence which may be the same or different. Therefore, the present process may be useful not only for producing large amounts of one target nucleic acid sequence, but also for amplifying simultaneously more than one different target nucleic acid sequence located on the same or different nucleic acid molecules.

The nucleic acids may be obtained from any source, for example: genomic or cDNA libraries, plasmids, cloned DNA or RNA, or from natural DNA or RNA from any source, including bacteria, yeast, viruses, and higher organisms such as plants or animals. The nucleic acid can be naturally occurring or synthetic, either totally or in part. Techniques for obtaining and producing the nucleic acids used in the present processes are well known to those skilled in the art. If the nucleic acid contains two strands, it is necessary to separate the strands of the nucleic acid before it can be used as the original template, either as a separate step or simultaneously with the synthesis of the primer extension products. Additionally, if the starting material is first strand DNA, second strand DNA may advantageously be created by processes within the purview of those skilled in the art and used as the original template from which the engineered template is created.

First strand cDNA and mRNA are particularly useful as the original template for the present methods. Suitable methods for generating DNA templates are known to and readily selected by those skilled in the art. In one embodiment, 1st strand cDNA is synthesized in a reaction where reverse transcriptase catalyzes the synthesis of DNA complementary to any RNA starting material in the presence of an oligodeoxynucleotide primer and the four deoxynucleoside triphosphates, dATP, dGTP, dCTP, and TTP. The reaction is initiated by the non-covalent bonding of the oligo-deoxynucleotide primer to the 3' end of mRNA followed by stepwise addition of the appropriate deoxynucleotides as determined by base pairing relationships with the mRNA nucleotide sequence, to the 3' end of the growing chain. As those skilled in the art will appreciate, all mRNA in a sample can be used to generate first strand cDNA through the annealing of oligo dT to the poly A tail of the mRNA.

Once the original template is obtained, a primer 20 and a boundary oligonucleotide 30 are annealed to the original template 10. (See Fig. 1.) A strand of nucleic acid complementary to the portion of the original template beginning at the 3' end of the primer up to about the 5' end of the boundary oligonucleotide is polymerized.

The primer 20 that is annealed to the original template includes a portion 25 that anneals to the original template and optionally a portion 22 of predetermined sequence that preferably does not anneal to the template, and optionally a restriction site 23 between portions 22 and 25. Thus, for example, where the original template is mRNA, a portion having a predetermined sequence that does not anneal to the template is not needed, but rather the primer can be any gene-specific internal sequence of the mRNA or oligo dT which will anneal to the unique poly A tail of the mRNA.

The primer anneals to the original template adjacent to the target sequence 12 to be amplified. It is contemplated that the primer can anneal to the original template upstream of the target sequence (or downstream in the case, e.g., of an mRNA original template) to be amplified, or that the primer may overlap the beginning of the target sequence 12 to be amplified as shown in Figure 1. The predetermined sequence of portion 22 of the primer is selected so as to provide a sequence to which the single primer used during the amplification process can hybridize as described in detail below. Preferably, the predetermined sequence is not native in the original template and does not anneal to the original template, as shown in Fig. 1. Optionally, the predetermined sequence may include a restriction site useful for insertion of

a portion of the engineered template into an expression vector as described more fully hereinbelow.

The boundary oligonucleotide 30 that is annealed to the original template serves to terminate polymerization of the nucleic acid. Any oligonucleotide capable of terminating nucleic acid polymerization may be utilized as the boundary oligonucleotide 30. In a preferred embodiment the boundary oligonucleotide includes a first portion 35 that anneals to the original template 10 and a second portion 32 that is not susceptible to an extension reaction. Techniques to prevent the boundary oligo from acting as a site for extension are within the purview of one skilled in the art. By way of example, portion 32 of the boundary oligo 30 may be designed so that it does not anneal to the original template 10 as shown in Fig. 1. In such embodiments, the boundary oligonucleotide 30 prevents further polymerization but does not serve as a primer for nucleic acid synthesis because the 3' end thereof does not hybridize with the original template 10. Alternatively, the 3' end of the boundary oligo 30 might be designed to include locked nucleic acid to achieve the same effect. Locked nucleic acid is disclosed for example in WO 99/14226, the contents of which are incorporated herein by reference. Those skilled in the art will envision other ways of ensuring that no extension of the 3' end of the boundary oligo occurs.

Primers and oligonucleotides described herein may be synthesized using established methods for oligonucleotide synthesis which are well known in the art. Oligonucleotides, including primers of the present invention include linear oligomers of natural or modified monomers or linkages, such as deoxyribonucleotides, ribonucleotides, and the like, which are capable of specifically binding to a target polynucleotide by way of a regular pattern of monomer-to monomer interactions such as Watson-Crick base pairing. Usually monomers are linked by phosphodiester bonds or their analogs to form oligonucleotides ranging in size from a few monomeric units e.g., 3-4, to several tens of monomeric units. A primer is typically single-stranded, but may be double-stranded. Primers are typically deoxyribonucleic acids, but a wide variety of synthetic and naturally occurring primers known in the art may be useful for the methods of the present disclosure. A primer is complementary to the template to which it is designed to hybridize to serve as a site for the initiation of synthesis, but need not reflect the exact sequence of the template. In such a case, specific hybridization of the primer to the

template depends on the stringency of the hybridization conditions. Primers may be labeled with, e.g., chromogenic, radioactive, or fluorescent moieties and used as detectable moieties.

Polymerization of nucleic acid can be achieved using methods known to those skilled in the art. Polymerization is generally achieved enzymatically, using a DNA polymerase or reverse transcriptase which sequentially adds free nucleotides according to the instructions of the template. The selection of a suitable enzyme to achieve polymerization for a given template and primer is within the purview of those skilled in the art. In certain embodiments, the criteria for selection of polymerases includes lack exonuclease activity or DNA polymerases which do not possess a strong exonuclease activity. DNA polymerases with low exonuclease activity for use in the present process may be isolated from natural sources or produced through recombinant DNA techniques. Illustrative examples of polymerases that may be used, are, without limitation, T7 Sequenase v. 2.0, the Klenow Fragment of DNA polymerase I lacking exonuclease activity, the Klenow Fragment of Taq Polymerase, exo.- Pfu DNA polymerase, Vent. (exo.-) DNA polymerase, and Deep Vent. (exo-) DNA polymerase.

In a particularly useful embodiment, the use of a boundary oligonucleotide is avoided by removing unneeded portions of the starting material by digestion. In this embodiment, which is shown schematically in Fig. 2A, a restriction oligonucleotide 70 is annealed to the starting material 100 at a preselected location. The restriction oligonucleotide provides a double stranded portion on the starting material containing a restriction site 72. Suitable restriction sites, include, but are not limited to Xho I , Spe I, Nhe1, Hind III, Nco I, Xma I, Bgl II, Bst I, and Pvu I. Upon exposure to a suitable restriction enzyme, the starting material is digested and thereby shortened to remove unnecessary sequence while preserving the desired target sequence 12 (or portion thereof) to be amplified on what will be used as the original template 110. Once the original template 110 is obtained, a primer 20 is annealed to the original template 110 (see Fig. 2B) adjacent to or overlapping with the target sequence 12 as described above in connection with previous embodiments. A strand of nucleic acid 40 complementary to the portion of the original template between the 3' end of the primer 20 and the 5' end of the original template 110 is polymerized. As those skilled in the art will appreciate, in this embodiment where a restriction oligonucleotide is employed to generate the original template, there is no need to use a boundary oligonucleotide, because primer extension can be allowed to proceed all the way to the 5' end of the shortened original template 110.

Once polymerization is complete (i.e., growing strand 40 reaches the boundary oligonucleotide 30 or the 5' end of the shortened original template 110), the newly synthesized complementary strand is separated from the original template by any suitable denaturing method including physical, chemical or enzymatic means. Strand separation may also be induced by an enzyme from the class of enzymes known as helicases or the enzyme RecA, which has helicase activity and in the presence of riboATP is known to denature DNA. The reaction conditions suitable for separating the strands of nucleic acids with helicases are described by Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLIII "DNA: Replication and Recombination" (New York: Cold Spring Harbor Laboratory, 1978), B. Kuhn et al., "DNA Helicases", pp. 63-67, and techniques for using RecA are reviewed in C. Radding, Ann. Rev. Genetics, 16:405-37 (1982).

The newly synthesized complementary strand thus includes sequences provided by the primer 20 (e. g., the predetermined sequence 22, the optional restriction site 23 and the annealing portion 25 of the primer) as well as the newly synthesized portion 45 that is complementary to the portion of the original template 10 between the location at which the primer 20 was annealed to the original template 10 and either the portion of the original template 10 to which the boundary oligonucleotide 30 was annealed or through to the shortened 5' end of the original template. See Fig. 3.

Optionally, multiple rounds of polymerization using the original template and a primer are performed to produce multiple copies of the newly synthesized complementary strand for use in subsequent steps. It is contemplated that 2 to 10 rounds or more (preferably, 15-25 rounds) of linear amplification can be performed when a DNA template is used. Making multiple copies of the newly synthesized complementary strand at this point in the process (instead of waiting until the entire engineered template is produced before amplifying) helps ensure that accurate copies of the target sequence are incorporated into the engineered templates ultimately produced. It is believed that multiple rounds of polymerization based on the original template provides a greater likelihood that a better representation of all members of the library will be achieved, therefore providing greater diversity compared to a single round of polymerization.

The next step in preparing the engineered template involves annealing a nested oligonucleotide 50 to the 3' end of the newly synthesized complementary strand, for example

as shown in Fig. 3. As seen in Fig. 3, the nested oligonucleotide 50 provides a template for further polymerization necessary to complete the engineered template. Nested oligonucleotide 50 includes a portion 52 that does not hybridize and/or includes modified bases to the newly synthesized complementary strand, thereby preventing the nested oligonucleotide from serving as a primer. Nested oligonucleotide 50 also includes a portion 55 that hybridizes to the 3' end of the newly synthesized complementary strand. Nested oligonucleotide 50 may optionally also define a restriction site 56. The final portion 58 of nested oligonucleotide 50 contains a hairpin structure. From the point at which portion 55 extends beyond the 3' end of the beginning the newly synthesized complementary strand, the nested oligonucleotide serves as a template for further polymerization to form the engineered template. It should be understood that the nested oligo may contain part of the target sequence (if part thereof was truncated in forming the original template) or may include genes that encode a polypeptide or protein (or portion thereof) such as, for example, one or more CDR's or Framework regions or constant regions of an antibody. It is also contemplated that a collection of nested oligonucleotides having different sequences can be employed, thereby providing a variety of templates which results in a library of diverse products. Thus, polymerization will extend the newly synthesized complementary strand by adding additional nucleic acid 60 that is complementary to the nested oligonucleotide as shown in Fig. 3. Techniques for achieving polymerization are within the purview of one skilled in the art. As previously noted, in selecting a suitable polymerase, an enzyme lacking exonuclease activity may be employed to prevent the 3' end of the nested oligo from acting as a primer. Because of hairpin structure 50 of the nested oligonucleotide, eventually the newly synthesized complementary strand will turn back onto portion 45 of the same strand which will then serve as the template for further polymerization. Polymerization will continue until the end of the primer is reached, at which point the newly synthesized strand will terminate with a portion whose sequence is complementary to the primer.

Once polymerization is complete, the engineered template 120 is separated from the nested oligonucleotide 50 by techniques well known to those skilled in the art such as, for example, heat denaturation. The resulting engineered template 120 contains a portion derived from the original primer 20, portion 45 that is complementary to a portion of the original template, and portion 65 that is complementary to a portion of the nested oligonucleotide and includes a hairpin structure 68, and a portion 69 that is complementary to portion 45. (See

Figs. 4A and B.) The 3' end of engineered template 120 includes a portion containing a sequence that is complementary to primer 20. Thus, for example, as shown in Fig. 4A, the 3' end of engineered template 120 includes portion 22' containing a sequence that is complementary to the predetermined sequence of portion 22 of primer 20. This allows for amplification of the desired sequence contained within engineered template 120 using a single primer having the same sequence as the predetermined sequence of primer portion 22 (or a primer that is complementary thereto) using techniques known to those of ordinary skill in the art.

As another example (shown in Fig. 4B), where mRNA is used as the template and oligo dT is used as the primer, the 3' end of engineered template 120 includes poly A portion that is complementary to the oligo dT primer. In this case, any sequence along portion 45 can be selected for use as the primer to be annealed to portion 69 once the engineered template is denatured for single primer amplification. Optionally, the primer may include a non-annealing portion, such as, for example, a portion defining a restriction site.

During single primer amplification, the presence of a polymerase having exonuclease activity is preferred because such enzymes are known to provide a "proofreading" function and have relatively higher processivity compared to polymerases lacking exonuclease activity.

Due to hairpin structure 68 there is internal self annealing between the 5' end predetermined sequence and the 3' end sequence which is complementary to the predetermined sequence on the engineered template. Upon denaturation and addition of a primer having the predetermined sequence, the primer will hybridize to the template and amplification can proceed.

After amplification is performed, the products may be detected using any of the techniques known to those skilled in the art. Examples of methods used to detect nucleic acids include, without limitation, hybridization with allele specific oligonucleotides, restriction endonuclease cleavage, single-stranded conformational polymorphism (SSCP), analysis.gel electrophoresis, ethidium bromide staining, fluorescence resonance energy transfer, hairpin FRET essay, and TaqMan assay.

Once the engineered nucleic acid is amplified a desired number of times, restriction sites 23 and 66 or any internal restriction site can be used to digest the strand so that the target nucleic acid sequence can be ligated into a suitable expression vector. The vector may then be

used to transform an appropriate host organism using standard methods to produce the polypeptide or protein encoded by the target sequence.

In particularly useful embodiments, the methods described herein are used to amplify target sequences encoding antibodies or portions thereof, such as, for example the variable regions (either light or heavy chain) using cDNA of an antibody. In this manner, a library of antibodies can be amplified and screened. Thus, for example, starting with a sample of antibody mRNA that is naturally diverse, first strand cDNA can be produced and digested to provide an original template. A primer can be designed to anneal upstream to a selected complementary determining region (CDR) so that the newly synthesized nucleic acid strand includes the CDR. By way of example, if the target sequence is heavy chain CDR3, the primer may be designed to anneal to the heavy chain framework one (FR1) region. Those skilled in the art will readily envision how to design appropriate primers to anneal to other upstream sites or to reproduce other selected targets within the antibody cDNA based on this disclosure.

The following Examples are provided to illustrate, but not limit, the present invention(s):

Example 1. Amplification of a repertoire of Ig kappa light chain variable genes

First strand cDNA synthesis

First strand cDNA to be used as the original template was generated from 2 μg of human peripheral blood lymphocyte (PBL) mRNA with an oligo-dT primer using the SuperScript II First Strand Synthesis Kit (Invitrogen) according to the manufacturer's instructions. The 1st strand cDNA product was purified over a QIAquick spin column (QIAGEN PCR Purification Kit) and eluted in 400 μL of nuclease-free water.

Second strand linear amplification (SSLA) in the presence of blocking oligonucleotide

The second strand cDNA reaction contained 5 μL of 1st strand cDNA original template, 0.5 μM primer JMX26VK1a, 0.5 μM blocking oligo CKLNA1, 0.2 mM dNTPs, 5 units of AmpliTaq Gold DNA polymerase (Applied Biosystems), 1x GeneAmp Gold Buffer(15 mM Tris-HCl, pH 8.0, 50 mM KCl), and 1.5 mM $MgCl_2$. The final volume of the reaction was 98 μL. The sequence of primer JMX26VK1a, which hybridizes to the framework 1 region of VK1a genes, was 5' GTC ACT CAC GAA CTC ACG ACT CAC GGA GAG CTC RAC ATC CAG ATG ACC CAG 3' (Seq. ID No. 1) where R is an equal mixture of A and G. The sequence of the blocking oligo CKLNA1, which hybridizes to the 5' end of the VK constant

region, was 5' GAA CTG TGG CTG CAC CAT CTG 3' (Seq. ID No. 2), where the underlined bases are locked nucleic acid (LNA) nucleotide analogues. After an initial heat denaturation step of 94°C for 3 minutes, linear amplification of 2nd strand cDNA was carried out for 20 cycles of 94°C for 15 seconds, 56°C for 15 seconds, and 68°C for 1 minute.

Nested Oligo Extension Reaction

After the last cycle of linear amplification, 2 µL of a nested/hairpin oligo designated "JK14TSHP" was added to give a final concentration of 20 µM. The sequence of JK14TSHP was 5' CCT TAG AGT CAC GCT AGC GAT TGA TTG ATT GAT TGATTG TTT GTG ACT CTA AGG TTG GCG CGC CTT CGT TTG ATY TCC ACC TTG GTC C(ps)T(ps)G(ps)P 3' (Seq. ID No. 3) where Y is an equal mixture of C and T and (ps) are phosphorothioate backbone linkages and P is a 3' propyl group. For nested oligo extension reaction, two cycles of 94°C for 1 minute, 56°C for 15 seconds, and 72°C for 1 minute were performed, followed by a 10 minute incubation at 72°C to allow complete extension of the hairpin. The reaction products were purified over a QIAquick spin column (QIAgen PCR Purification Kit) and eluted in 50 µL of nuclease-free water.

Analysis of engineered template

The efficiency of the nested oligo extension reaction was determined by amplifying the products with either a primer set specific for the engineered product or a primer set that detects all VK1a/JK14 second strand cDNA products (including the engineered product). For specific detection of engineered product, a 10 µL aliquot was amplified for 20 or 25 cycles with primers designated "JMX26" and "TSDP". Primer JMX26 hybridizes to the 5' end of JMX26VK1a, the framework 1 primer used in the second strand cDNA reaction. Primer TSDP hybridizes to the hairpin-loop sequence added to the 3' ends of the second strand cDNAs in the nested oligo extension reaction. The sequence of primer JMX26 was 5' GTC ACT CAC GAA CTC ACG ACT CAC GG 3' (Seq. ID No. 4). The sequence of primer TSDP was 5' CAC GCT AGC GAT TGA TTG ATT G 3' (Seq. ID No. 5). For detection of all VK1a/JK14 second strand cDNA products a 10 µL aliquot was amplified for 20 or 25 cycles with primers JMX26 and JK14. The sequence of primer JK14, which hybridizes to the framework 4 region of JK1 and JK4 genes, was 5' GAG GAG GAG GAG GAG GAG GGC GCG CCT GAT YTC CAC CTT GGT CCC 3' (Seq. ID No. 6). Both reactions contained 1x GeneAmp Gold Buffer, 1.5 mM $MgCl_2$, 7.5% glycerol, 0.2 mM dNTPs, and 0.5 µM of each primer in a final volume of 50 µL.

The results with primers JMX26 and TSDP demonstrated the successful production of nested oligo and extended VK stem-loop DNA when using SSLA DNA that was blocked specifically with a boundary oligo. Suitable controls showed that when using the nested oligo in the presence of SSLA DNA that was not blocked, only a minimal amount of amplified product was produced. Additional controls without the nested oligo were negative. However, VK1a/JK14 second strand cDNA products were detected equally among all tested samples.

Single primer amplification of the stem-loop cDNA template

Conditions that were previously shown to amplify a 352bp stem-15bp loop DNA product were as follows: 10 pg of the stem-loop DNA, 2 μM primer, 50 mM Tris-HCl, pH 9.0, 1.5 mM $MgCl_2$, 15 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, 1.7 M betaine, 0.2 mM dNTPs, and 2.5 units of Z-Taq DNA Polymerase (Takara Shuzo) in a final volume of 50 μL. The thermal cycling conditions were an initial denaturation step of 96°C for 2.5 minutes, 35 cycles of 96°C for 30 seconds, 64°C for 30 seconds, 74°C for 1.5 minutes, and a final extension step of 74°C for 10 minutes. Oligonucleotides containing the modified bases 5-methyl-2'-deoxycytidine and/or 2-amino-2'-deoxyadenosine have been shown to prime much more efficiently than unmodified oligonucleotides at primer binding sites located within hairpin structures (Lebedev et al. 1996. *Genetic Analysis: Biomolecular Engineering* 13, 15-21). These modifications work by increasing the melting temperature of the primer, allowing the annealing step of the amplification to be performed at a higher temperature. JMX26 primers containing ten 5-methyl-2'-deoxycytidines or seven 2-amino-2'-deoxyadenosines have been synthesized.

Cloning VK products

Amplified fragments are cloned by Sac I / Asc I into an appropriate expression vector that contains, in frame, the remaining portion of the kappa constant region. Suitable vectors include pRL5 and pRL4 vectors (described in U.S. Provisional Application 60/254,411, the disclosure of which is incorporated herein by reference), fdtetDOG, PHEN1, and pCANTAB5E. Individual kappa clones can be sequenced.

Expanding the repertoire of VKappa amplified products

Further coverage of the VK repertoire is achieved by using the above protocols with a panel of primers for the generation of the second strand DNA. The primers contain JMX26 sequence, a Sac I restriction site, and a region that anneals to 1st strand cDNA in the framework 1 region of human antibody kappa light chain genes. The antibody annealing sequences were

derived from the VBase database primers (www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html) which were designed based on the known sequences of human antibodies and are reported to cover the entire human antibody repertoire of kappa light chain genes. Below is a list of suitable primers:

JMX26Vk1a (Seq. ID No.7)
GTCACTCACGAACTCACGACTCACGGAGAGCTCRACATCCAGATGACCCAG

JMX26Vk1b (Seq. ID No.8)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGMCATCCAGTTGACCCAG

JMX26Vk1c (Seq. ID No.9)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGCCATCCRGATGACCCAG

JMX26Vk1d (Seq. ID No.10)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGTCATCTGGATGACCCAG

JMX26Vk2a (Seq. ID No.11)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGATATTGTGATGACCCAG

JMX26Vk2b (Seq. ID No.12)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGATRTTGTGATGACTCAG

JMX26Vk3a (Seq. ID No.13)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATTGTGTTGACRCAG

JMX26Vk3b (Seq. ID No.14)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATAGTGATGACGCAG

JMX26Vk3c (Seq. ID No.15)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATTGTAATGACACAG

JMX26Vk4a (Seq. ID No.16)

GTCACTCACGAACTCACGACTCACGGAGAGCTCGACATCGTGATGACCCAG

JMX26Vk5a (Seq. ID No.17)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAACGACACTCACGCAG

JMX26Vk6a (Seq. ID No.18)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGAAATTGTGCTGACTCAG

JMX26 Vk6b (Seq. ID No.19)
GTCACTCACGAACTCACGACTCACGGAGAGCTCGATGTTGTGATGACACAG

In the foregoing sequences, R is an equal mixture of A and G, M is an equal mixture of A and C, Y is an equal mixture of C and T, W is an equal mixture of A and T, and S is an equal mixture of C and G.

Example 2. Amplification of a repertoire of IgM or IgG heavy chain or lambda light chain variable genes

Similar protocols are applied to the amplification of both heavy chain and lambda light chain genes. JMX26, or another primer without antibody specific sequences, is used for each of those applications. If JMX26 is used, the second strand DNA is generated with the primers listed below which contain JMX26 sequence, a restriction site (Sac I for lambda, Xho I for heavy chains), and a region that anneals to 1st strand cDNA in the framework 1 region of human antibody lambda light chain or heavy chain genes. The antibody annealing sequences were derived from the VBase database primers (www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html) which were designed based on the known sequences of human antibodies and are reported to cover the entire human antibody repertoire of lambda light chain and heavy chain genes.

Lambda light chain Framework 1 Specific Primers:

JMX26VL1a (Seq. ID No. 20)
GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGTGCTGACTCAG

JMX26VL1b (Seq. ID No. 21)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGTGYTGACGCAG

JMX264VL1C (Seq. ID No. 22)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGTCGTGACGCAG

JMX26VL2 (Seq. ID No. 23)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGTCTGCCCTGACTCAG

JMX26VL3a (Seq. ID No. 24)

GTCACTCACGAACTCACGACTCACGGAGAGCTCTCCTATGWGCTGACTCAG

JMX26VL3b (Seq. ID No. 25)

GTCACTCACGAACTCACGACTCACGGAGAGCTCTCCTATGAGCTGACACAG

JMX26VL3c (Seq. ID No. 26)

GTCACTCACGAACTCACGACTCACGGAGAGCTCTCTTCTGAGCTGACTCAG

JMX26VL3d (Seq. ID No. 27)

GTCACTCACGAACTCACGACTCACGGAGAGCTCTCCTATGAGCTGATGCAG

JMX26VL4 (Seq. ID No. 28)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGCYTGTGCTGACTCAA

JMX26VL5 (Seq. ID No. 29)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGSCTGTGCTGACTCAG

JMX26VL6 (Seq. ID No. 30)

GTCACTCACGAACTCACGACTCACGGAGAGCTCAATTTTATGCTGACTCAG

JMX26VL7 (Seq. ID No. 31)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGRCTGTGGTGACTCAG

JMX26VL8 (Seq. ID No. 32)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGACTGTGGTGACCCAG

JMX26VL4/9 (Seq. ID No. 33)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCWGCCTGTGCTGACTCAG

JMX26VL10 (Seq. ID No. 34)

GTCACTCACGAACTCACGACTCACGGAGAGCTCCAGGCAGGGCTGACTCAG

In the foregoing sequences (and throughout this disclosure), R is an equal mixture of A and G, M is an equal mixture of A and C, Y is an equal mixture of C and T, W is an equal mixture of A and T, and S is an equal mixture of C and G.

Heavy Chain Framework 1 Specific Primers:

JMX24VH1a (Seq. ID No. 35)

GTCACTCACGAACTCACGACTCACGGActcgagCAGGTKCAGCTGGTGCAG

JMX24VH1b (Seq. ID No. 36)

GTCACTCACGAACTCACGACTCACGGActcgagCAGGTCCAGCTTGTGCAG

JMX26VH1c (Seq. ID No. 37)

GTCACTCACGAACTCACGACTCACGGActcgagSAGGTCCAGCTGGTACAG

JMX26VH1d(Seq. ID No. 38)

GTCACTCACGAACTCACGACTCACGGActcgagCARATGCAGCTGGTGCAG

JMX26VH2a (Seq. ID No. 39)

GTCACTCACGAACTCACGACTCACGGActcgagCAGATCACCTTGAAGGAG

JMX26VH2b (Seq. ID No. 40)

GTCACTCACGAACTCACGACTCACGGActcgagCAGGTCACCTTGARGGAG

JMX26VH3a (Seq. ID No. 41)

GTCACTCACGAACTCACGACTCACGGActcgagGARGTGCAGCTGGTGGAG

JMX26VH3b (Seq. ID No. 42)

GTCACTCACGAACTCACGACTCACGGActcgagCAGGTGCAGCTGGTGGAG

JMX26VH3c (Seq. ID No. 43)

GTCACTCACGAACTCACGACTCACGGActcgagGAGGTGCAGCTGTTGGAG

JMX26VH4a (Seq. ID No. 44)

GTCACTCACGAACTCACGACTCACGGActcgagCAGSTGCAGCTGCAGGAG

JMX26VH4b (Seq. ID No. 45)

GTCACTCACGAACTCACGACTCACGGActcgagCAGGTGCAGCTACAGCAG

JMX26VH5a (Seq. ID No. 46)

GTCACTCACGAACTCACGACTCACGGActcgagGARGTGCAGCTGGTGCAG

JMX26VH6a (Seq. ID No. 47)

GTCACTCACGAACTCACGACTCACGGActcgagCAGGTACAGCTGCAGCAG

JMX26VH7a (Seq. ID No. 48)

GTCACTCACGAACTCACGACTCACGGActcgagCAGGTSCAGCTGGTGCAA

In the foregoing sequences (and throughout this disclosure), R is an equal mixture of A and G, K is an equal mixture of G and T, and S is an equal mixture of C and G.

Blocking oligos for the constant domain of IgM, IgG, and lambda chains are designed. Essentially, a region downstream of that required for cloning the genes is identified, and within that region, a sequence useful for annealing a blocking oligo is determined. For example with IgG heavy chains, a native Apa I restriction site present in the CH1 domain can be used for cloning. Generally, the boundary oligo is located downstream of that native restriction site.

Compatible nested oligos are then designed which contained all the elements described previously.

Once amplified, the lambda light chain genes are cloned as is described above for the kappa light chain genes. Likewise, amplified IgG heavy chain fragments are cloned by Xho I / Apa I into an appropriate expression vector that contains, in frame, the remaining portion of the CH1 constant region. Suitable vectors include pRL5, pRL4, fdtetDOG, PHEN1, and pCANTAB5E. Amplified IgM heavy chain fragments are cloned by Xho I / EcoR I into an appropriate expression vector that contains, in frame, the remaining portion of the CH1 constant region. Like the Apa I present natively in IgG genes, the EcoR I site is native to the IgM CH1 domain. Libraries co-expressing both light chains and heavy chains can be screened or selected for Fabs with the desired binding activity.

Example 3

Amplification of a Repertoire of Human IgM Heavy Chain Genes

First Strand cDNA Synthesis

Human peripheral blood lymphocyte (PBL) mRNA was used as the original template to generate the first strand cDNA with ThermoScript RT-PCR System (Invitrogen Life Technologies). In addition to oligo dT primer, a phosphoramidate oligonucleotide (synthesized by Annovis Inc. Aston, PA) was also included in the reverse transcription reaction. The phosphoramidate oligonucleotide serves as a boundary for reverse transcriptase. The first strand cDNA synthesis was terminated at the location where the phosphoramidate oligonucleotide anneals with the mRNA. The phosphoramidate oligonucleotide, PN-1, was designed to anneal with the framework I region of immunoglobulin (Ig) heavy chain VH3 genes and PN-VH5 was designed to anneal with the framework I region of all the Ig heavy chain genes. A control for first strand cDNA synthesis was also set up by not including the phosphoramidate blocking oligonucleotide. The first strand cDNA product was purified by QIAquick PCR Purification Kit (QIAGEN).

Phosphoramidate Framework I Blocking Oligonucleotides for Ig Heavy Chain Genes have the following sequences:

PN-1 5' GCCTCCCCCAGACTC 3' (Seq. ID No. 49)

PN-VH5 5' GCTCCAGACTGCACCAGCTGCAC(C/T)TCGG 3' (Seq. ID No. 50)

Examination of the Blocking Efficiency

The blocking efficiency in first strand cDNA synthesis was examined by PCR reactions using blocking check primers and primer CM1, dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, and the first strand cDNA synthesis product. PCR was performed on a PTC-200 thermal cycler (MJ Research) by heating to 94^{0}C for 30 seconds and followed by cycles of 94^{0}C for 15 second, 60^{0}C for 15 second, and 72^{0}C for one minute. The blocking check primers were designed to anneal with the leader sequences of Ig heavy chain genes. The sequence of CM1, which hybridizes with the CH1 region of IgM, was 5' GCTCACACTAGTAGGCAGCTCAGCAATCAC 3' (Seq. ID No. 51). Blocking was analyzed by gel electrophoresis of the PCR products. With appropriate number of cycles, less PCR product was observed from the reverse transcription reactions containing the blocking oligonucleotides than the one does not contain the blocking oligonucleotides, an indication that termination of first strand cDNA synthesis was provided by the hybridization of the blocking oligonucleotides.

The sequences of the blocking check Primers for Ig heavy chain genes have the following sequences:

H1/7blck	5' C TGG ACC TGG AGG ATC C 3'	(Seq. ID No. 52)
H1blck2	5' C TGG ACC TGG AGG GTC T 3'	(Seq. ID No. 53)
H1blck3	5' C TGG ATT TGG AGG ATC C 3'	(Seq. ID No. 54)
H2blck	5' GACACACTTTGCTCCACG 3'	(Seq. ID No. 55)
H2blck2	5' GAC ACA CTT TGC TAC ACA 3'	(Seq. ID No. 56)
H3blck	5' TGGGGCTGAGCTGGGTTT 3'	(Seq. ID No. 57)
H3blck2	5' TG GGA CTG AGC TGG ATT T 3'	(Seq. ID No. 58)
H3blck3	5' TT GGG CTG AGC TGG ATT T 3'	(Seq. ID No. 59)
H3blck4	5' TG GGG CTC CGC TGG GTT T 3'	(Seq. ID No.60)
H3blck5	5' TT GGG CTG AGC TGG CTT T 3'	(Seq. ID No. 61)
H3blck6	5' TT GGA CTG AGC TGG GTT T 3'	(Seq. ID No. 62)
H3blck7	5' TT TGG CTG AGC TGG GTT T 3'	(Seq. ID No. 63)
H4blck	5' AAACACCTGTGGTTCTTC 3'	(Seq. ID No. 64)
H4blck2	5' AAG CAC CTG TGG TTT TTC 3'	(Seq. ID No. 65)
H5blck	5' GGGTCAACCGCCATCCT 3'	(Seq. ID No. 66)

H6blck 5' TCTGTCTCCTTCCTCATC 3' (Seq. ID No. 67)

Second Strand cDNA Synthesis And Nesting Oligonucleotide Extension Reaction:

The purified first strand cDNA synthesis product was used in a nested oligo extension reaction with a hairpin-containing nesting oligonucleotide , dNTPs, Advantage-2 DNA polymerase mix (Clontech), and the reaction buffer. The extension reaction was performed with a GeneAmp PCR System 9700 thermocyler (PE Applied Biosystems). It was heated to 94^0C for 30 seconds and followed by ten cycles of 94^0C for 15 seconds, appropriate annealing temperature for each nesting oligonucleotide for 15 seconds, ramping the temperature to 90^0C at 10% of the normal ramping rate, and 90^0C for 30 seconds. The resulted heavy chain gene should contain a hairpin structure.

Nesting Oligonucleotides for Ig VH1 Heavy Chain genes had the following sequences:

hpVH1-1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAGGTGCAGCTGGTGCAG TCTGGGGCT GAGGTGAAGAAGCCTG AAG 3' (Seq. ID No. 68)

hpVH1-2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGCAGaTGCAGCTGGTGCAG TCTGGGGCTGAGGTGAAGAAGaCTAAT 3' (Seq. ID No. 69)

hpVH1-3

5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATG CAG CTG GTG CAG TCT GGGCCT GAG GTG AAG AAG CCT ATT 3' (Seq. ID No. 70)

hpVH1-4

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGCAG TCTGGGGCTGAGGTGAAGAAGCCTGAAG 3' (Seq. ID No. 71)

Nesting Oligonucleotides for Ig VH2 Heavy Chain Genes:

hpVH2-1

5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATC ACC TTG AAG GAG TCT GGT CCT ACG CTG GTG AAA CCC ACATAA 3' (Seq. ID No. 72)

hpVH2-2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTC ACC TTG AAG GAG TCT GGT CCT GYG CTG GTG AAA CCC AC TAA 3' Y:C/T (Seq. ID No. 73)

Nesting Oligonucleotides for Ig VH3 Heavy Chain Genes:

hpVH3A1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GAG TCT GGG GGA GGC TTG GT(C/A) CAG CCT GGGAAA 3' C/A: M(Seq. ID No. 74)

hpVH3A2

5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGTCTGGG GGAGGC(T/C)TGGT(A/C)AAGCCTGGGAAA 3' (Seq. ID No. 75)

hpVH3A3

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGT CTGGGGGAGGTGTGGTACGGCCTGGGAAA 3' (Seq. ID No. 76)

hpVH3A4

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGA CTGGAGGAGGCTTGATCCAGCCTGGGAAG 3' (Seq. ID No. 77)

hpVH3A5

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGT CTGGGGGAGTCGTGGTACAGCCTGGGAAA 3' (Seq. ID No. 78)

hpVH3A6

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGGAGT CT CGGGGAGTCTTGGTACAGCCTGGGAAA 3' (Seq. ID No. 79)

hpVH3A7

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTG GTA CAG CCT GGCAAA 3' (Seq. ID No. 80)

hpVH3A8

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTG GTC CAG CCT GGAAAA 3' (Seq. ID No. 81)

hpVH3A9

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTA GTT CAG CCT GGGAAA 3' (Seq. ID No. 82)

hpVH3A10

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GA G TCT GGG GGA GGC TTG GTA CAG CCA GGGAAA 3' (Seq. ID No. 83)

ots-hp-VH3b

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGCAGGTGCAGCTGGTGGAGT CTGGGGGAGGCGTGGTCCAGCCTGGGTTT 3' (Seq. ID No. 84)

hp-VH3B2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGCAGGTGCAGCTGGTGGAGT CTGGGGGAGGCTTGGTCAAGCCTGGAAAG 3' (Seq. ID No. 85)

hpVH3C

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTGTTG GA G TCT GGG GGA GGC TTG GTA CAG CCT GGGAAA 3' (Seq. ID No. 86)

Nesting Oligonucleotides for Ig VH4 Heavy Chain Genes:

hpVH4-1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG STG CAG CTG CAG GA G TCG GGC CCA GGA CTG GTG AAG CCT T AAA 3' S: C/G (Seq. ID No. 87)

hpVH4-2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG CTG CAG CTG CAG GAG TCG GGC TCA GGA CTG GTG AAG CCT T AAA 3' (Seq. ID No. 88)

hpVH4-3

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG AG GTG CAG CTG CAGCAG TGG GGC GCA GGA CTG TTG AAG CCT T AAT 3' (Seq. ID No. 89)

Nesting Oligonucleotides for Ig VH5 Heavy Chain Genes:

othpVH52

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAGCTGGTGCAGT CT GGAGCAGAGGTGAAAAAGCCCGGGGAAAA 3' (Seq. ID No. 90)

Nesting Oligonucleotides for Ig VH6 Heavy Chain Genes:

hpVH6

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTA CAG CTG CAG CAG TCA GGT CCA GGA CTG GTG AAG CCC AAA 3' (Seq. ID No. 91)

Nesting Oligonucleotides for Ig VH7 Heavy Chain Genes:

hpVH7

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTG CAG CTG GTG CAA TCT GGG TCT GAG TTG AAG AAG CCT ATA 3' (Seq. ID No. 92)

Additional Ig Heavy Chain Nesting Oligonucleotides:

hpVH 3kb1

5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCGACTGGTGGAG TCTGGGGGAGACTTGGTAGAACCGGGGAAG 3' (Seq. ID No. 93)

hpVH 3kb2

5'CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGATGCAACTGGTGGAG TCTGGGGGAGCCTTCGTCCAGCCGGGGAAG 3' (Seq. ID No. 94)

Single Primer Amplification of IgM Hairpin-Containing Fd Fragments

Products from the nesting oligo extension reaction (i.e. the engineered template) were amplified using Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, dNTPs, and a single primer named CM3 primer. The sequence for the CM3 primer, which anneals with the CH1 region of IgM, was:

5' AGAATTTGACTAGTTGGCAAGAGGCACGTTCTTTTCTTTGTTGCCGT 3' (Seq. ID No. 95).

The amplification reaction was performed with a GeneAmp PCR System 9700 thermocyler (PE Applied Biosystems). It was initially heated to 94^{0}C for 30 seconds and followed by thirty to forty cycles of 94^{0}C for 15 seconds, appropriate annealing temperature for 15 seconds, ramping the temperature to 90^{0}C at 10% of the normal ramping speed, and at 90^{0}C for 30 seconds. The amplified product was examined by electrophoresis to be of the expected size, ~ 0.7 kb. The amplified fragments were cloned into an expression vector and their sequences were confirmed to be human IgM.

Example 4

Amplification of a Repertoire of Human IgG Heavy Chain Genes from a Donor Immunized with Hepatitis B Surface Antigen

First Strand cDNA Synthesis

The same protocol as example 3 is employed using mRNA of PBL from a human donor immunized with hepatitis B surface antigen and the phosphoramidate boundary oligonucleotides designed to anneal with the leader sequence of the Ig heavy chain genes. The phosphoramidate leader boundary oligonucleotides for Ig heavy chain genes have the following sequences:

PNVH3ld 5' CACCTCACACTGGACACCTTT 3' (Seq. ID No. 95)

PNVH4ld	5' CTGGGACAGGACCCATCTGGG 3'	(Seq. ID No. 96)
PNVH1ld	5' TGGGAGTGGGCACCTGTGG 3'	(Seq. ID No. 97)
PNVH2ld	5' CTGGGACAAGACCCATGAAG 3'	(Seq. ID No. 98)
PNVH5ld	5' TCGGAACAGACTCCTTGGAGA 3'	(Seq. ID No. 99)
PNVH6ld	5' CTGTGACAGGACACCCCATGG 3'	(Seq. ID No. 100)

Examination of the Blocking Efficiency

The blocking efficiency in first strand cDNA synthesis is examined by PCR reactions using dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, the first strand cDNA synthesis product, the blocking check primers in Example 3, and the pooled primer mixture of CG1Z, CG2speI, CG3speI, and CG4SpeI. The sequence of primer CG1Z, which hybridized with the CH1 region of IgG1, is 5' GCATGTACTAGTTTTGTCACAAGATTTGGG 3'. (Seq. ID No. 101) The sequence of primer CG2speI, which hybridized with the CH1 region of IgG2, is 5'AAGGAAACTAGTTTTGCGCTCAACTGTCTTGTCCACCTT 3'. (Seq. ID No. 102) The sequence of primer CG3speI, which hybridized with the CH1 region of IgG3, is 5'AAGGAAACTAGTGTCACCAAGTGGGGTTTTGAGCTC 3'. (Seq. ID No. 103) The sequence of primer CG4speI, which hybridized with the CH1 region of IgG4, is 5'AAGGAAACTAGTACCATATTTGGACTCAACTCTCTTG 3'. (Seq. ID No. 104) PCR is performed on a PTC-200 thermal cycler (MJ Research) by heating to 94^0C for 30 seconds before the following cycle is run, 94^0C for 15 second, 60^0C for 15 second, and 72^0C for one minute. The PCR products were analyzed by gel electrophoresis. With appropriate number of cycles less PCR products were observed from reverse transcription reactions containing the blocking oligonucleotide than the one does not contain blocking oligonucleotide, an indication that termination of first strand cDNA synthesis was provided by hybridization of the leader *boundary oligonucleotides.*

Second Strand cDNA Synthesis And Nesting Oligonucleotide Extension Reaction:

The same protocol as Example 3 is employed with nesting oligonucleotides having the following sequences are used.

Nesting Oligonucleotides for Ig Heavy Chain VH3 Genes:

HpH3L1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGSAGGTGCAGCTGGTGGAG TCYGAAA 3' where S is an equal mixture of C and G, and Y is an equal mixture of T and C (Seq. ID No. 105)

HpH3L2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAGGAGGTGCAG CTG TTG GAG TCT GAAT 3' (Seq. ID No. 106)

HpH3L3

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GAG ACT GATA 3' (Seq. ID No. 107)

HpH3L4

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG GAG TCT CAAA 3' (Seq. ID No. 108)

Nesting Oligonucleotides for Ig Heavy Chain VH4 Genes:

HpH4L1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG STG CAG CTG CAG GAG TCG GAAA 3' where S is an equal mixture of C and G (Seq. ID No. 109)

HpH4L2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG CTG CAG CTG CAG GAG TCC AAA 3' (Seq. ID No. 110)

HpH4L3

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTG CAG CTA CAG CAG TGG GAAA 3' (Seq. ID No. 111)

Nesting Oligonucleotides for Ig Heavy Chain VH1 Genes:

HpH1L1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTB CAG CTK GTG CAG AAA 3' where B is an equal mixture of C, G and T and K is an equal mixture of G and T (Seq. ID No. 112)

HpH1L2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG SAG GTC CAG CTG GTA CAG AAA 3' where S is an equal mixture of C and G (Seq. ID No. 113)

HpH1L3

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATG CAG CTG GTG CAG AAA 3' (Seq. ID No. 114)

HpH1L4

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAA ATG CAG CTG GTG CAG AAA 3' (Seq. ID No. 115)

Nesting Oligonucleotides for Ig Heavy Chain VH2 Genes:

HpH2L1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG ATC ACC TTG AAG GAG TCT AAA 3' (Seq. ID No. 116)

HpH2L2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTC ACC TTG AAG GAG TCT AAA 3' (Seq. ID No. 117)

Nesting Oligonucleotides for Ig Heavy Chain VH5 Genes:

HpH5L1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAG GTG CAG CTG GTG CAG AAA 3' (Seq. ID No. 118)

HpH5L2

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG GAA GTG CAG CTG GTG CAG AAA 3' (Seq. ID No. 119)

Nesting Oligonucleotides for Ig Heavy Chain VH6 Genes:

HpH6L1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTA CAG CTG CAG CAG TC AAA 3' (Seq. ID No. 120)

Nesting Oligonucleotides for Ig Heavy Chain VH7 Genes:

HpH7L1

5' CTCGAGGGCCCGCGAAAGCGGGCCCTCGAG CAG GTG CAG CTG GTG CAA TAAA 3' (Seq. ID No. 121)

Single Primer Amplification of Human IgG Heavy Chain Fd Hairpin Containing Fragments

The sample protocol as Example 3 was employed using CG1Z, CG2speI, CG3speI, or CG4SpeI as the primer.

Cloning of Amplified IgG Heavy Chain Fd Fragments into a Phage Display Vector

The amplified IgG heavy chain fd hairpin fragments are analyzed by gel electrophoresis. The ~0.7 kb fragment is separated from the primers by cutting out the gel slice and the DNA was collected by electroelution. The eluted DNA was precipitated by ethanol and resuspended in water. It is digested with restriction enzymes XhoI and SpeI and purified by the QIAquick PCR Purification Kit (QIAGEN). The purified XhoI-SpeI fragment is ligated into a suitable plasmid into which the light chain kappa genes amplified from the same donor had previously been cloned. The ligated reaction was transformed into *E. coli XL-1 Blue* strain {F' *proA*$^+$*B*$^+$ *lacI*^{q} Δ *(lacZ) M15 Tn10 / recA1 endA1 gyrA96 thi-1 hsdR17 supE44 relA1 lac*} by electroporation.

Selection of Human IgG Antibodies That Bind with The Hepatitis B Surface Antigen

The XL-1 Blue cells electroporated with the ligation reaction of the phagemid vector and the heavy chain Fd fragments were grown in SOC medium at 37^{0}C with shaking for one hour. SOC medium is 20 mM glucose in SB medium which contains 1% MOPS hemisodium salt, 3% Bacto Tryptone, and 2% Bacto Yeast Extract. Cells transformed with the plasmid were selected by adding carbenicillin to the culture and they were grown for two hours before infected with a helper phage, VCSM13. After two hours XL-1 Blue cells infected with the helper phage were selected by adding Kanamycin to the culture and the infected cells were amplified overnight by growing at 37^{0}C with shaking. The next morning the amplified phages were harvested by precipitating with polyethylene glycol (PEG) from the culture supernatant. The PEG precipitated phages were collected by centrifugation. They were resuspended in 1% bovine serum albumin (BSA) in TBS buffer and used in panning for selecting human IgG antibodies that bind with the hepatitis B surface antigen. The resuspended phages were bound with the hepatitis B surface antigen immobilized on the ELISA plate (Costar). The unbound phages were washed off with a washing buffer (0.5% Tween 20 in PBS) and the bound phages were eluted off the plate with a phage elution buffer (0.1M HCl / glycine, pH 2.2, 1 mg/ml BSA) and neutralized with a neutralization buffer (2M Tris Base). The eluted phages were infected with *E. coli* ER strain {F' *proA*$^+$*B*$^+$ *lacI*^{q} Δ *(lacZ) M15 /fhuA2 (ton A) Δ (lac-proAB) supE thi-1 Δ (hsdMS-mcrB) 5}*, followed by infection with VCSM13 helper phage. The panning procedure for selecting antibodies bound to hepatitis B surface antigen were repeated three more times.

ELISA Screening of Antibody Clones That Bind with The Hepatitis B Surface Antigen

Phages eluted at the fourth round of panning were infected with *E. coli* Top10F' strain {F' *lacI*^{q} ,Tn10 (Tet ^{R} mcrA *Δ (mrr-hsdRMS-mcrBC) Φ8(lacZ Δm15 Δlacx74 deoR recA1 araD13 Δ(ara-leu)7697 galU galK [sL(Str^{R}) endA1 nupG*) and plated on LB-agar plates containing carbenicilin and tetracycline. Individual clones were picked from the plates and grown overnight in SB medium containing carbenicilin and tetracycline. The IgG Fab fragment will be secreted into the culture supernatant. The next morning cells were removed from these cultures by centrifugation and the culture supernatant was screened in ELISA assay for binding to hepatitis B surface antigen immobilized on the ELISA plates. To reduce false positives the ELISA plates were pre-blocked with BSA before binding with the Fab fragments in culture supernatant. The non-binding Fab fragments were washed off by a washing solution (0.05% Tween 20 in PBS). Following the wash, plates were incubated with anti-human IgG $(Fab')_2$ conjugated with alkaline phosphatase (Pierce) which reacts with p-Nitrophenyl phosphate (Sigma), a chromogenic substrate that shows absorbance at OD405. Positive binding clones were identified by a plate reader (Bio RAD Model 1575) with light absorbance at OD405. Among the ninety-four clones screened there were twenty-eight positive clones.

Characterization of the Hepatitis B Surface Antigen Binding Clones

The IgG heavy chain genes of positive clones from ELISA screening were characterized by DNA sequencing. Plasmid DNA was extracted from the positive clones and sequenced using primers leadVHpAX, NdP, or SeqGZ (Retrogen, San Diego, CA). The sequencing primers have the following sequences:

VBVH3A 5' GAGCCGCACGAGCCCCTCGAGGARGTGCAGCTGGTGGAG 3' (Seq. ID No. 122)

VBVH 3B 5' GAGCCGCACGAGCCCCTCGAGGAGGTGCAGCTGGTGGAG 3' (Seq. ID No. 123)

VBVH 3C 5' GAGCCGCACGAGCCCCTCGAGGAGGTGCAGCTGTTGGAG 3' (Seq. ID No. 124)

VBVH 4A 5' GAGCCGCACGAGCCCCTCGAGCAG(CG)TGCAGCTGCAGGAG 3' (Seq. ID No. 125)

VBVH 4B 5' GAGCCGCACGAGCCCCTCGAGCAGGTGCAGCTACAGCAG 3' (Seq. ID No. 126)

LeadVHPAX 5' GCGGCGCAGCCGGCGATGGCG 3' (Seq. ID No. 127)

NdP 5' AGCGTAGTCCGGAACGTCGTACGG (Seq. ID No. 128)

SeqGZ 5' GAAGTAGTCCTTGACCAG 3' (Seq. ID No. 129)

The sequences of the variable region of these IgG heavy chain genes from nineteen positive clones are shown in Figure 5. The great diversity of these IgG heavy chain genes shows this method can efficiently amplify the repertoire of human IgG heavy chain genes from immunized donors.

EXAMPLE 5

Amplification of a Repertoire of Human Light chain Kappa Genes

First Strand cDNA Synthesis

The same protocol as example 3 is employed using the phosphoramidate boundary oligonucleotides designed to hybridize with the leader sequence of the kappa light chain genes. The phosphoramidate leader boundary oligonucleotides for kappa light chain genes have the following sequences:

PNK1ld: 5' T GTC ACA TCT GGC ACC TGG 3' (Seq. ID No. 130)

PNK2ld: 5' TC CCC ACT GGA TCC AGG GAC 3' (Seq. ID No. 131)

PNK3ld: 5' C TCC GGT GGT ATC TGG GAG 3' (Seq. ID No. 132)

PNK4ld: 5' TC CCC GTA GGC ACC AGA GA 3' (Seq. ID No. 133)

PNK5ld: 5' TC TGC CCT GGT AT C AGA GAT 3' (Seq. ID No. 134)

PNK6ld: 5' C ACC CCT GGA GGC TGG AAC 3' (Seq. ID No. 135)

Examination of the Blocking Efficiency

The blocking efficiency in first Strand cDNA Synthesis was examined by PCR reactions using blocking check primers and primer CK1DX2, dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, and the first strand cDNA synthesis product. PCR was performed on a PTC-200 thermal cycler (MJ Research) by heating to 94^0C for 30 seconds and followed by cycles of 94^0C for 15 second, 60^0C for 15 second, and 72^0C for one minute. The blocking check primers were designed to anneal with the leader sequences of kappa light chain genes. The sequence of CK1DX2, which hybridizes with the constant region of Kappa light chain, was

5'AGACAGTGAGCGCCGTCTAGAATTAACACTCTCCCCTGTTGAAGCTCTTTGTGAC

GGGCGAACTCAG 3'. (Seq. ID No. 136) Blocking was analyzed by gel electrophoresis of the PCR products. With appropriate number of cycles less PCR products was observed from reverse transcription reactions containing the blocking oligonucleotide than one that does not contain blocking oligonucleotide, an indication that termination of first strand cDNA synthesis was provided by hybridization of the leader boundary oligonucleotides.

Blocking Check Primers for Kappa Light Chain Genes have the following sequences:

K1blck: 5' CTCCGAGGTGCCAGATGT 3' (Seq. ID No. 137)

K1 /2blck2: 5' GCT CAG CTC CTG GGG CT 3'(Seq. ID No. 138)

K2blck: 5' GTCCCTGGATCCAGTGAG 3' (Seq. ID No. 139)

K3blck: 5' CTCCCAGATACCACCGGA 3' (Seq. ID No. 140)

K3blck2: 5' GCG CAG CTT CTC TTC CT 3' (Seq. ID No. 141)

K3blck3: 5' CAC AGC TTC TTC TTC CTC 3' (Seq. ID No. 142)

K4blck: 5' ATCTCTGGTGCCTACGGG 3'(Seq. ID No. 143)

K5blck: 5' ATCTCTGATACCAGGGCA 3' (Seq. ID No. 144)

K6blck: 5' GTTCCAGCCTCCAGGGGT 3' (Seq. ID No. 145)

Second Strand cDNA Synthesis And Nesting Oligonucleotide Extension Reaction:

The same protocol as Example 3 is employed using nesting oligonucleotides having the following sequences:

Nesting oligonucleotides for Light Chain Kappa Vk1:

HpK1L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GMC ATC CAG ATG ACC CAG TCT CCTAA 3' wherein M is an equal mixture of A and C (Seq. ID No. 146)

HpK1L2

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC AAC ATC CAG ATG ACC CAG TCT CC TAA 3' (Seq. ID No. 147)

HpK1L3

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GMC ATC CAG TTG ACC CAG TCT CC TAA 3' wherein M is an equal mixture of A and C (Seq. ID No. 148)

HpK1L4

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GCC ATC CGG ATG ACC CAG TCT CCTAT 3' (Seq. ID No. 149)

HpK1L5

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GTC ATC TGG ATG ACC CAG TCT CCTAT 3'. (Seq. ID No. 150)

Nesting oligonucleotides for Light Chain Kappa Vk2:

HpK2L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAT ATT GTG ATG ACC CAG ACT CTTA 3' (Seq. ID No. 151)

HpK2L2

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAT GTT GTG ATG ACT CAG TCT CC TAA 3' (Seq. ID No. 152)

HpK2L3

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAT ATT GTG ATG ACT CAG TCT CCTAA3' (Seq. ID No. 153)

Nesting oligonucleotides for Light Chain Kappa Vk3:

HpK3L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATT GTG TTG ACG CAG TCT CCTAA3' (Seq. ID No. 154)

HpK3L2

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATA GTG ATG ACG CAG TCT CCTAA3' (Seq. ID No. 155)

HpK3L3

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATT GTA ATG ACA CAG TCT CCTAA3' (Seq. ID No. 156)

Nesting oligonucleotides for Light Chain Kappa Vk4:

HpK4L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAC ATC GTG ATG ACC CAG TCT CCTAT3' (Seq. ID No. 157)

Nesting oligonucleotides for Light Chain Kappa Vk5:

HpK5L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ACG ACA CTC ACG CAG TCT CCTAA3' (Seq. ID No. 158)

Nesting oligonucleotides for Light Chain Kappa Vk6:

HpK6L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC GAA ATT GTG CTG ACT CAG TCT CCTAT3' (Seq. ID No. 159)

Single Primer Amplification of Kappa Hairpin Fragments

The same protocol as Example 3 is employed using CK1DX2 as the primer.

EXAMPLE 6

Amplification of a Repertoire of Human Light Chain Lambda Genes

First Strand cDNA Synthesis

The same protocol as example 3 is employed using the following phosphoramidate boundary oligonucleotides designed to hybridize with the leader sequence of the lambda light chain genes. The phosphoramidate boundary oligonucleotides for lambda light chain genes have the sequences:

PNL1ld: 5' CTG GGC CCA GGA CCC TGT GC 3' (Seq. ID No. 160)

PNL2ld: 5' CTG GGC CCA GGA CCC TGT 3'. (Seq. ID No. 161)

PNL3ld: 5' GA GGC CAC AGA GCC TGT GCA GAG AGT GAG 3' (Seq. ID No. 162)

PNL4ld1: 5' CAG AGC ACA GAG ACC TGT GGA3' (Seq. ID No. 163)

PNL4ld2: 5' CTG GGA GAG AGA CCC TGT CCA3' (Seq. ID No. 164)

PNL5ld1: 5' CTG GGA GAG GGA ACC TGT GCA3' (Seq. ID No. 165)

PNL6ld1: 5' ATT GGC CCA AGA ACC TGT GCA3' (Seq. ID No. 166)

PNL7ld1: 5' CTG AGA ATT GGA CCC TGG GCA3' (Seq. ID No. 167)

PNL8ld1: 5' CTG AGA ATC CAC TCC TGA TCC3' (Seq. ID No. 168)

PNL9ld1: 5' CTG GGA GAG GGA CCC TGT GAG3' (Seq. ID No. 169)

PNL10ld1: 5' CTG GAC CAC TGA CAC TGC AGA3' (Seq. ID No. 170)

Examination of the Blocking Efficiency

The same protocol as example 3 is employed using the following blocking check primers and primer CL2DX2, dNTPs, Advantage-2 DNA polymerase mix (Clontech), the reaction buffer, and the first strand cDNA synthesis product. The blocking check primers have the following sequences:

L1blck: 5' CAC TGY GCA GGG TCC TGG 3' (Seq. ID No. 171)

L2blck: 5' CAG GGC ACA GGG TCC TGG 3' (Seq. ID No. 172)

L3blck1: 5' TAC TGC ACA GGA TCC GTG 3' (Seq. ID No. 173)

L3blck2: 5' CAC TTT ACA GGT TCT GTG 3' (Seq. ID No. 174)

L3blck3: 5' TTC TGC ACA GTC TCT GAG 3' (Seq. ID No. 175)

L3blck4: 5' CTC TGC ACA GGC TCT GAG 3' (Seq. ID No. 176)

L3blck5: 5' CTT TGC TCA GGT TCT GTG 3' (Seq. ID No. 177)

L3blck6: 5' CAC TGC ACA GGC TCT GTG 3' (Seq. ID No. 178)

L3blck7: 5' CTC TAC ACA GGC TCT ATT 3' (Seq. ID No. 179)

L3blck7: 5' CTC TGC ACA GTC TCT GTG3' (Seq. ID No. 180)

L4blck1: 5' TTC TCC ACA GGT CTC TGT 3' (Seq. ID No. 181)

L4blck2: 5' CAC TGG ACA GGG TCT CTC 3' (Seq. ID No. 182)

L5blck1: 5' CAC TGC ACA GGT TCC CTC 3' (Seq. ID No. 183)

L6blck: 5' CAC TGC ACA GGT TCT TGG 3' (Seq. ID No. 184)

L7blck: 5' TGC TGC CCA GGG TCC AAT 3' (Seq. ID No. 185)

L8blck: 5' TAT GGA TCA GGA GTG GAT 3' (Seq. ID No. 186)

L9blck: 5' CTC CTC ACA GGG TCC CTC 3' (Seq. ID No. 187)

L10blck: 5' CAC TCT GCA GTG TCA GTG 3' (Seq. ID No. 188)

The sequence of CL2DX2, which hybridizes with the CL region of Lambda genes, has this sequence: 5' AGACAGTGACGCCGTCTA GAATTATGAACATTCTGTAGG 3' (Seq. ID No. 189).

<u>Second Strand cDNA Synthesis And Nesting Oligonucleotide Extension Reaction:</u>

The same protocol as Example 3 is employed using the nesting oligonucleotides having the following sequences:

Nesting oligonucleotides for Lambda Light Chain VL1:

HpL1L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG TCT GTG CTG ACT CAG CCA CCAAA 3' (Seq. ID No. 190)

HpL1L2

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG TCT GTG YTG ACG CAG CCG CCAAA 3' (Seq. ID No. 191)

Nesting oligonucleotides for Lambda Light Chain VL2:

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG TCT GCC CTG ACT CAG CCT SAAA3' (Seq. ID No. 192)

Nesting oligonucleotides for Lambda Light Chain VL3:

HpL3L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ACT CAG CCA CYAAA3' (Seq. ID No. 193)

HpL3L2

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ACA CAG CYA CCAAT 3' (Seq. ID No. 194)

HpL3L3

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC T CT TCT GAG CTG ACT CAG GAC CCAAA 3' (Seq. ID No. 195)

HpL3L4

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GTG CTG ACT CAG CCA CCAAA 3' (Seq. ID No. 196)

HpL3L5

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ATG CAG CCA CCAAA 3' (Seq. ID No. 197)

HpL3L6

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC TCC TAT GAG CTG ACA CAG CCA TCAAA3' (Seq. ID No. 198)

Nesting oligonucleotides for Lambda Light Chain VL4:

HpL4L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CTG CCT GTG CTG ACT CAG CCC CCAAA3' (Seq. ID No. 199)

HpL4L2

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CCT GTG CTG ACT CAA TCA TCAAA3' (Seq. ID No. 200)

HpL4L3

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CTT GTG CTG ACT CAA TCG CCAAA3' (Seq. ID No. 201)

Nesting oligonucleotides for Lambda Light Chain VL5:

HpL5L1 5e. 5b

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CCT GTG CTG ACT CAG CCA YCAAA3' (Seq. ID No. 202)

HpL5L2 5c

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG GCT GTG CTG ACT CAG CCG GCAAA3' (Seq. ID No. 203)

Nesting oligonucleotides for Lambda Light Chain VL6:

HpL6L1 6a

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC AAT TTT ATG CTG ACT CAG CCC CAAAA3' (Seq. ID No. 204)

Nesting oligonucleotides for Lambda Light Chain VL7 and VL8:

HpL7/8L1

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG ACT GTG GTG ACY CAG GAG CCAAA3' (Seq. ID No. 205)

HpL7L2

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC G CAG GCT GTG GTG ACT CAG GAG CCAAA3' (Seq. ID No. 206)

Nesting oligonucleotides for Lambda Light Chain VL9:

HpL9L

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG CCT GTG CTG ACT CAG CCA CCAAA3' (Seq. ID No. 207)

Nesting oligonucleotides for Lambda Light Chain VL10:

5'GAGCTCGGCCCGCGAAAGCGGGCCGAGCTC CAG GCA GGG CTG ACT CAG CCA CCAAA3' (Seq. ID No. 208)

Single Primer Amplification of Lambda Hairpin Containing Fragments

The same protocol as Example 3 is employed using CL2DX2 as the primer.

Example 7

Amplification of a Repertoire of Human IgG Heavy Chain Genes from a Donor Immunized with Hepatitis B Surface Antigen

First Strand cDNA Synthesis

The same protocol as example 3 was employed using mRNA of PBL from a human donor immunized with hepatitis B surface antigen as the original template using blocking oligonucleotides that anneal to FR1 of the variable heavy chain.

Examination of the Blocking Efficiency

The same protocol as example 4 was employed.

Second Strand cDNA Synthesis And Nesting Oligonucleotide Extension Reaction:

The same protocol as Example 3 was employed.

Single Primer Amplification of Human IgG Heavy Chain Fd Hairpin Containing Fragments

The sample protocol as Example 4 was employed.

Cloning of Amplified IgG Heavy Chain Fd Fragments into a Phage Display Vector

The sample protocol as Example 4 was employed.

Selection of Human IgG Antibodies That Bind with The Hepatitis B Surface Antigen

The sample protocol as Example 4 was employed.

ELISA Screening of Antibody Clones That Bind with The Hepatitis B Surface Antigen

The sample protocol as Example 4 was employed. Among the ninety-four clones screened eighty clones are positive.

Characterization of the Hepatitis B Surface Antigen Binding Clones

The sample protocol as Example 4 was employed. Sequences of the variable regions of the heavy chain genes from fourteen positive clones are listed in Figure 6. The sequence diversity of these clones and others produced shows this method can efficiently amplify the repertoire of human heavy chain genes from immunized donors.

It will be understood that various modifications may be made to the embodiments described herein. Therefore, the above description should not be construed as limiting, but merely as exemplifications of preferred embodiments. Those skilled in the art will envision other modifications within the scope and spirit of this disclosure.